

**REMARKS**

This application is amended in a manner to place it in condition for allowance at the time of the next Official Action.

**Status of the Claims**

Claims 10-13 are amended. Claims 19-30 are new. Support for the amendment to the claims may be found, for example, at page 6, line 9 to page 7, line 15, page 9, lines 29-36, page 10, lines 10-24, and the Examples.

Claims 14-18 have been cancelled.

Claims 10-13 and 19-30 remain in this application.

**Claim Rejections-35 USC §112**

Claims 10-18 were rejected under 35 U.S.C. §112, first paragraph, for not complying with the enablement requirement. This rejection is respectfully traversed for the reasons below.

The position of the Official Action was that the present application would not give enough information demonstrating that methods for switching off the gene of starch phosphorylase other than TDNA insertion would enable to implement the invention.

Claims 10 and 11 have now been amended to specify that the gene coding for starch phosphorlyase is switched off by introducing a mutation in the genome of said plant.

Accordingly, the use of techniques of switching off a gene other than mutation techniques, such as siRNA or ribozymes expression, is now excluded from the scope of the claims, which renders moot the objection of lack of enablement concerning the use of such techniques.

The Example of the application presents the best embodiment of the invention which is implemented using T-DNA insertional mutagenesis technique in order to switch off the gene coding for starch phosphorylase. The plant thus obtained is the DDS72 line, wherein the gene coding for starch phosphorylase is mutated by T-DNA insertion as shown in SEQ ID NO: 2.

The position of the Official Action also was that the present application would not give guidance as to the mechanism by which the enzyme activity is repressed in the DDS72 mutant line.

As known from the one skilled in the art, and for example explained in Krysan *et al.* (1999) *Plant Cell* 11 :2283-2290, included in the appendix of this amendment, the insertion of a piece of T-DNA on the order of 5 to 25 kb in length produces a dramatic disruption of gene function. Accordingly, it would have been readily apparent to one of ordinary skill in the art that the presence of T-DNA in the starch phosphorylase gene sequence, as described in SEQ ID NO: 2, induces a disruption of the gene function and of the protein level and activity.

Moreover, experimental results obtained by one of the inventors Mr. Christophe d'Hulst confirm that the DDS72 mutant line does not display any starch phosphorylase activity. These experimental results are provided in the Declaration Under Rule 132 included in the appendix of this amendment.

Additionally, these experimental results further demonstrate that the phenotype of increased size of starch grains and of increased starch content described in the present application is only due to the decrease of the starch phosphorylase level in the plant. Indeed, they show that other enzymes, which could have an impact on the size of starch grains or on starch content in the plant, display the same activity in the DDS72 line as in the wild type line.

Accordingly, any technique enabling obtaining a disruption of the gene coding for starch phosphorylase, and hence a reduced activity of starch phosphorylase, as defined in amended claims 10 and 11, is suitable to achieve the present invention. Such techniques are well-known from the one skilled in the art.

Therefore, for the above reasons it is believed that the present claims do comply with the enablement requirement, and withdrawal of the rejection is respectfully requested.

Claims 10 and 13 were rejected under 35 U.S.C. §112, second paragraph, for being indefinite for omitting essential steps. This rejection is respectfully traversed.

Claim 10 is amended to positively recite an active method step.

Therefore, withdrawal of the rejection is respectfully requested.

**Claim Rejections-35 USC §102**

Claims 10, 11 and 13-18 were rejected under 35 U.S.C. §102(b) as being anticipated by KOSSMANN et al. U.S. 6,686,514 (KOSSMANN). This rejection is respectfully traversed for the reasons below.

KOSSMANN describes the production of transgenic maize wherein starch phosphorylase activity is reduced by introducing an antisense nucleic acid, in order to obtain a modified synthesis of starch. The nature of the starch modification is not specified, but it is described as a modification in the physical and chemical properties, in particular in the amylose/amylopectin ratio, in the degree of branching, in the average chain length, in the phosphate content and in the size and/or the shape of starch grains (column 6, lines 20 to 25).

Amended claims 10 and 11 define, respectively, a method for increasing the size of starch grains and the starch content of a plant or of a plant part, and a method for obtaining plants or plant parts that produce starch grains of increased size and with higher starch content. The methods comprise the step of

switching off the gene coding for starch phosphorylase by introducing a mutation in the genome of said plant.

KOSSMANN does not describe a method as defined in claim 10 or 11 comprising a step of switching off the gene coding for starch phosphorylase by introducing a mutation in the genome of the plant. Indeed, KOSSMANN only describes plants with an elevated or reduced activity of different starch associated enzymes. In particular, plants with a reduced activity of a starch associated enzyme are only achieved by the expression of an antisense RNA (column 8, lines 51 to 54). Additionally, KOSSMANN cites several starch-associated enzymes and several types of starch modification, but KOSSMANN fails to associate, unambiguously, a plant with a reduced activity of starch phosphorylase with a phenotype of increased size of starch grains and of higher starch content.

Therefore, KOSSMANN fails to anticipate independent claims 10 and 11, and dependent claims 12 and 13, as well as new dependent claims 19-25 and new method claim 30, which requires the method of claim 11, and withdrawal of the rejection is respectfully requested. For similar reasons, KOSSMANN fails to anticipate new claims 26-30.

#### **Claim Rejections-35 USC §103**

Claims 10-18 were rejected under 35 U.S.C. §103(a) as being unpatentable over CRITCHLEY et al. 2001 The Plant Journal

26: 89-100 (CRITCHLEY) in view of KOSSMANN. This rejection is respectfully traversed for the reasons below.

The position of the Official Action was that it would have been obvious for one of ordinary skill in the art in view of these documents to modify the method of screening an increased accumulation of starch using the T-DNA insertional mutagenesis as described in CRITCHLEY, to study the effect of switching off the starch phosphorylase taught by KOSSMANN to screen for increased starch accumulation. In support of this position the Official Action stated that one of ordinary skill in the art would have been motivated to do so because starch phosphorylase would have been identified as an enzyme that would modify starch content by both KOSSMANN and CRITCHLEY.

The subject-matter of the present claims relates to methods enabling to increase both the starch content and the size of starch grains in plants.

CRITCHLEY describes the inactivation of D-enzyme using T-DNA. In particular, CRITCHLEY demonstrates that D-enzyme would be necessary for normal starch degradation (page 90, left column, §3). CRITCHLEY further discloses that the inactivation of D-enzyme, while being associated with a starch-excess phenotype, also induces a slight increase of starch phosphorylase activity (page 92, right column, §1). Nevertheless, CRITCHLEY does not identify starch phosphorylase as modifying the starch content.

On the contrary, in view of CRITCHLEY, one of ordinary skill in the art would have associated an increase in starch content with an activation of starch phosphorylase. Thus, CRITCHLEY teaches away from the claimed invention which links the inactivation of starch phosphorylase to an increase of the starch content and of the size of starch grains in a plant.

Accordingly, one of ordinary skill in the art would not have been motivated to inactivate the gene of starch phosphorylase in view of CRITCHLEY to increase the starch content and the size of starch grains in plants.

Furthermore, this conclusion is also supported by SMITH et al. 2003 (SMITH), cited in the IDS. SMITH indicates that the loss of expression of the phosphorylase has no general impact on leaf carbohydrate metabolism because this loss in the phosphorolytic pathway would be compensated by the activity of the hydrolytic pathway (page 580, right column, § 1). Thus, as evidenced by SMITH, the state of the art taught away from the invention which shows that starch phosphorylase inactivation is sufficient to obtain an increase of starch content and of the size of starch grains.

KOSSMANN fails to remedy the shortcomings of CRITCHLEY for reference purposes. As discussed relative to the anticipation rejection, in the transgenic maize of KOSSMANN, starch phosphorylase activity is reduced by introducing an antisense

nucleic acid. This reduction of the starch phosphorylase activity would enable obtaining a modified synthesis of starch.

However, this modification is not specified, but only described as a modification in the physical and chemical properties, in particular in the amylose/amylopectin ratio, in the degree of branching, in the average chain length, in the phosphate content or in the size and/or the shape of starch grains (column 6, lines 20 to 25).

Accordingly, KOSSMANN does not identify any modulation of a determined starch-modifying enzyme as having an impact on both the size of starch grains and the starch content of a plant. In particular, KOSSMANN does not suggest that the inactivation of starch phosphorylase could induce both an increase of the size of starch grains and an increase of the starch content. Indeed, as described page 3, lines 3 to 7 of the present application, the increase of the size of starch grains is not necessarily linked to the increase of the starch content.

Additionally, as disclosed in the present application (page 1, line 37 - page 2, line 2), previous studies (in particular SONNEWALD et al. (1995) *Plant. Mol. BioI.* 27:567-576 (SONNEWALD) reported that reduced expression of starch phosphorylase in leaves, by antisense repression (which corresponds to the techniques used in KOSSMANN), did not have a significant influence on the accumulation of starch in potatoes. Accordingly, this conclusion of SONNEWALD, the state of the art

further supported the conclusion that KOSSMANN teaches away from the claimed invention.

Thus, one of ordinary skill in the art would not have been motivated to switch off the gene of starch phosphorylase of CRITCHLEY in view of KOSSMANN, and given the state of the art, to increase both the starch content and the size of starch grains in plants.

Therefore, claims 10-13 and 19-30 are not rendered obvious by the proposed combination, and withdrawal of the rejection is respectfully requested.

### **Conclusion**

In view of the amendment to the claims, the evidence and declaration provided in the appendix, along with the foregoing remarks, this application is in condition for allowance at the time of the next Official Action. Allowance and passage to issue on that basis is respectfully requested.

Should there be any matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned at the telephone number listed below.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to our credit card which is being paid online

simultaneously herewith for any additional fees required under 37  
C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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**APPENDIX:**

The Appendix includes the following item(s):

- Krysan *et al.* (1999) *Plant Cell* 11 :2283-2290,
- a 37 CFR 1.132 Declaration of Christophe D'HULST, and
- CV of Christophe D'HULST